510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k040711

B. Analyte:
Anti-neutrophil cytoplasmic antibody (ANCA)

C. Type of Test:
Semi-quantitative, ELISA

D. Applicant:
RhiGene, Inc.

E. Proprietary and Established Names:
MESACUP Test PR-3

F. Regulatory Information:

1. Regulation section:
   21 CFR §866.5660 Multiple Antibodies Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MOB: Anti-neutrophil cytoplasmic antibodies (ANCA)
4. Panel:
   IM 82

G. Intended Use:

1. Intended use(s):
   The MESACUP Test PR-3 is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of IgG anti-proteinase III (PR-3) antibodies in human serum.

   Clinical (hospital and reference) laboratory personnel are the intended users of the MESACUP Test PR-3.
2. Indication(s) for use:
   The MESACUP Test PR-3 is indicated for *in vitro* use as an aid in the diagnosis of certain systemic vasculitides such as Wegener's granulomatosis.
3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument Requirements:
   None

H. Device Description:

The device is an enzyme-linked immunosorbent assay (ELISA) in which microtiter plates are used as the solid phase. The plate wells are coated with PR-3 antigens (native purified), which allow anti-PR-3 antibodies to react with the immobilized antigen (sample). The conjugate is polyclonal goat anti-human IgG (heavy chain specific) horseradish peroxidase (HRP), which uses 3,3'5,5' tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H_2O_2) as substrate. The kit contains two levels of calibrators (i.e., 0 units/mL and 100 u/mL) for interpretation of results.

Positive and negative control sera are included with the kit, which also contains sample diluent, wash buffer concentrate and stop solution.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   The Binding Site Bindazyme Human Anti-PR-3 Enzyme Immunoassay Kit
2. Predicate K number(s):
   k981029
3. Comparison with predicate:

Similarities		
Item	**MESACUP Test PR-3**	**Predicate**
Indications for Use	For detection of IgG anti-PR-3 antibodies as an aid in the diagnosis of certain systemic vasculitides such as Wegener's granulomatosis. [Note: The claim for the predicate device also includes the wording: *"certain forms of autoimmune vasculitis"*].	
Assay principle	Indirect ELISA	
Analyte	IgG anti-PR-3 antibodies	
Sample matrix	Serum	
Substrate	One-component TMB	
Differences		
Item	**MESACUP Test PR-3**	**Predicate**
Cut-off	23 U/mL	3.5 U/mL
Detection range	5 - 200 U/mL	1.23 - 100 U/mL
Assay time	150 minutes at Room t^{o}	90 minutes at Room t^{o}
Absorbance	450 nm / 620 nm	450 nm
Conjugate	HRP-goat anti-human IgG	HRP-rabbit anti-human IgG

J. Standard/Guidance Document Referenced (if applicable):
Not applicable

K. Test Principle:
The assay involves enzyme-linked immunosorbent assay (ELISA) technology. Calibrators and patient sera are incubated with PR-3 antigens (native purified) for a specified time, and then washed. This step is followed by incubation with horseradish peroxidase conjugated anti-human IgG. The reaction is then washed, stopped, and the color is allowed to develop and measured photometrically.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
   Three lots of the MESACUP Test PR-3 were used to determine the intra-assay, inter-assay and inter-lot value precision for the test.

   Intra-assay
   Intra-assay precision (% CV) was determined by running 2 serum samples (i.e., low positive and high positive) using one dilution and 40 replicates on 3 separate assays (3 separate lots). Three separate plates were randomly selected from each plate-coating run (kit-lot).

The mean % CV for intra-assay precision for the samples tested on 3 plates from each lot was 3.8% (Range: 3.1 – 4.3%).

Inter-assay, intra-lot
To determine the amount of variability between plates of the same lot, 2 samples in duplicate were tested on 7 separate assays from the same plate lot for each one of 3 separate plate lots of the device. The mean % CV for inter-assay, intra-lot precision was 8.6% with a range of 2.7 – 12.9%.

Inter-assay, inter-lot
The precision between lots was determined by comparing the values recovered for 2 different samples on 3 different kit lots. Each of the samples was tested in 40 replicates on one plate from each lot. The mean inter-assay, inter-lot % CV was 6.5%.

b. Linearity/assay reportable range:
The reportable range of 5-200 U/mL was demonstrated by recovery studies. Linearity is not claimed for this assay.

c. Traceability (controls, calibrators, or method):
An international reference material for anti-PR-3 antibodies is not available. The assay is calibrated in relative arbitrary units (U/mL).

d. Detection limit:
Not applicable.

e. Analytical specificity:
Several substances were added to each of three patient specimens, i.e., negative, low, and high, to test for interference. The results summarized below show that the addition of these substances at the levels tested does not affect the assay results.

Substance	Level Range (U/mL)	Negative			Low			High		
		Mean (U/mL)	***SD***	***%CV***	***Mean (U/mL)***	***SD***	***%CV***	***Mean (U/mL)***	***SD***	***%CV***
Hemoglobin	0 – 440	9.4	0.4	4.3	33.5	0.8	2.5	59.2	3.6	6.2
Bilirubin C	0 – 19.5	10.4	0.3	3.0	33.9	0.9	2.5	60.9	3.2	5.2
Bilirubin F	0 – 18.6	10.0	0.4	3.6	33.5	1.2	3.6	60.2	1.7	2.8
Chyle	0 – 2350	9.5	0.4	3.9	34.1	1.5	4.3	62.5	1.7	2.8
Rheumatoid Factor	0 - 500	10.0	0.7	7.2	32.8	1.6	5.0	59.4	4.2	7.1

f. Assay cut-off:
Sera from patients with systemic vasculitis (n= 78) were tested by indirect immunofluorescence (IIF) for the presence of anti-neutrophil cytoplasmic antibodies (ANCA). Positive samples were classified as either cytoplasmic or perinuclear ANCA. The results of testing the same samples with the MESACUP Test PR-3 were compared to those from p-ANCA IIF positives since "*the major binding agent for positive p-ANCA antibody specimens"* in the IIF method is proteinase-III. The best performance was observed at an assay cut-

off of 23 U/mL, i.e., 87.1% (27/31) of IIF pANCA+ were also MESACUP Test PR-3 +. There is no equivocal (gray) zone for this assay.

g. *Stability:*
Three manufacturing lots of the device were used in real time stability studies. The kits were stored at 2 – 8 ^{o}C. Six samples,, i.e., of the two *"calibrators and from a panel of PR-3 reactive patient specimens,"* were tested at 0, 4, 5, 6, 9, and 11 months, and evaluated for optical density (O.D.) value recovery (only the PR-3 reactive panel samples) per the established assay specifications. The kits were stable up to 11 months under the specified experimental conditions.

2. Comparison studies:
   a. *Method comparison with predicate device:*
   The tables below shows the results of comparison of serum samples (N=158) that were tested with the MESACUP Test PR-3 and the predicate device.

	Bindazyme +	**Bindazyme -**
MESACUP-PR-3 +	**27**	**0**
MESACUP-PR-3 -	**0**	**131**

STATISTIC	**Value**	**95% CI**
Prevalence	0.1709	0.1122 - 0.2296
Positive Agreement	1.0000	1.0000 – 1.0000
Negative Agreement	1.0000	1.0000 – 1.0000
Total Agreement	1.0000	1.0000 – 1.0000

   b. *Matrix comparison:*
   Serum is the only recommended matrix.

3. Clinical studies:
   a. *Clinical sensitivity:*
   Clinical sensitivity for the MESACUP-2 Test PR-3 was determined by testing sera (n = 78) from patients suspected to have a systemic vasculitis disease. Using 23 U/mL as the cut-off, 35% (27/78) of the samples were positive for anti-PR-3 antibodies. The mean value for this sample population was 36.9 U/mL (SD = $\pm$ 57.7). Single Factor ANOVA analysis that compared this value to the mean for the healthy controls gives a p-value of 2.55×10^{-7}. Therefore, at a level of $p<0.05$ for statistical significance, the results of this population were determined to be statistically different compared to the healthy controls.
   b. *Clinical specificity:*

The applicant evaluated samples, in duplicate, from 80 consecutive healthy blood donors and used these samples as the normal population. The mean value was 2.1 U/mL (SD = ± 3.1). None of the samples tested positive in this sample population. Therefore, the specificity was 100 % (80/80).

Similar studies were performed with samples from patients from various autoimmune diseases. The table below provides a summary of the results obtained in these subgroups.

Disease or Disease Status	N	Mean (U/mL)	Specificity (%)
Rheumatoid Arthritis	9	3.6	0
Sjögren's Syndrome	10	2.3	0
Systemic Lupus Erythematosus	10	5.6	10%
Mixed Connective Tissue Disease	10	2.2	0
Polymyositis/ Dermatomyositis	10	3.6	0

The absence of significant cross-reactivity demonstrates good specificity of the test for the intended indication

c. Other clinical supportive data (when a and b are not applicable):
Not applicable.

4. Clinical cut-off:
   See assay cut-off.
5. Expected values/Reference range:
   The expected value in the normal population is negative.

M. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.